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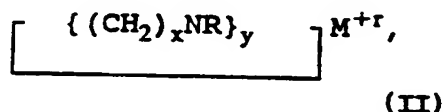
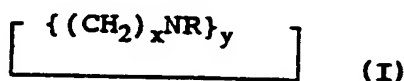
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(54) Title: POLYAZAMACROCYCLIC COMPOUNDS FOR COMPLEXATION OF METAL IONS



(57) Abstract

The present invention relates to a new polyazamacrocyclic compound or a salt thereof and its uses as a tissue specific chelator. The compound has formula (I), where x is 2, 3 or a combination of p 2(s) and q 3(s) where p + q = y; y is 3 or 4; R is (CH₂)_zP(=O)OR¹OR²; R¹ is H or CH₃; R² is C_nH_{1+2n}; n is 4 to 6; z is 1 to 3. In one important embodiment, this compound may be complexed with a metal to be a polyazamacrocyclic compound-metal complex having formula (II), where r is 2 or 3; and M is a metal ion, including a lanthanide, a heavy metal, or a radionuclide metal.

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DESCRIPTION
POLYAZAMACROCYCLIC COMPOUNDS FOR
COMPLEXATION OF METAL IONS

5

BACKGROUND OF THE INVENTION

Field of the Invention

10 The present invention relates to compositions and methods for enhancing contrast in imaging internal structures and functions of living subjects.

Imaging Modalities

15 Imaging of internal structures and functions of living subjects may be accomplished by applying electromagnetic radiation from external sources (as in conventional x-rays and computerized axial tomography) or internal sources (as in PET or positron emission tomography and radionuclide scans). Use of ionizing
20 radiation is avoided in imaging with nuclear magnetic resonance (NMR) and untrasonography, making these methods advantageous for many applications.

25 Whatever the imaging modality, consideration is given to means of increasing image contrast through localization of contrast agents in the region to be imaged. Such agents are frequently metals which emit, absorb, or scatter energy or, as in the case with NMR agents, increase the image signal strength locally. For
30 best effect, agents must be localized. This may be accomplished, for example, by direct injection of contrast agent (as in myelograms or retrograde urethrograms), through metabolic uptake of an agent (as in PET), and by conjugation of contrast agents with
35 monoclonal antibodies which tend to accumulate in certain tissues. The latter process in particular has been used

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in NMR image enhancement with chelated metal ions. Though well known, the process has several shortcomings:

- 1 - preparation of the antibody is complex;
- 5 2 - diminished immunoreactivity of the antibody occurs following conjugation;
- 3 - there is limited uptake of the conjugate by the target tissue; and
- 10 4 - there may be unfavorable interactions between the chelated ion and the antibody.

Because of the advantages of NMR imaging (good resolution and avoidance of ionizing radiation), an NMR contrast agent capable of greater localization would be clinically important. Such an agent would offer significant advantages over contrast agents of the prior art.

NMR Contrast Agents

20 The quality of the images obtained from an NMR scan is based on two properties: the proton densities of the various tissues and differences in proton relaxation rates. The proton density of tissues cannot be readily altered. Proton relaxation rates can be adjusted by
25 adding a paramagnetic relaxation agent, more commonly known as a "contrast agent." Contrast agents enhance the contrast in NMR images between magnetically similar but histologically dissimilar tissues.

30 Gadolinium, which has strong paramagnetic properties because of its seven unpaired electrons, has been tested as a contrast agent. It has a large magnetic moment which efficiently relaxes magnetic nuclei and increases tissue contrast in the region of the gadolinium.

35 One drawback of gadolinium as a contrast agent is its toxicity to animals, although a possible remedy for

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this problem is incorporation of gadolinium in a compound that would pass through the body and be excreted without releasing toxic gadolinium ions. Unfortunately, the rare earth elements (including gadolinium) do not
5 form stable covalent bonds with organic molecules, so such molecules can decompose in vivo and release the toxic ions.

Thus, there is a need for effective contrast agents
10 which avoid the toxicity problems inherent in using gadolinium or another metal ion. Further, it is desirable that a contrast agent control or influence the distribution of chelated ions in the body.

15 A even more desirable approach to the site-specific delivery of metal ions would be through use of stable chelates having inherent affinity for various tissue types. Inherent tissue affinity built into the organic chelating agent through modifications in both ionic
20 charge and degree of lipophilic character would offer substantial advantages over currently available agents.

SUMMARY OF THE INVENTION

25 The present invention relates to a series of new phosphorous-containing triaza- and tetraazamacrocyclic chelators which have inherent affinity for certain tissues. Following intravascular injection, chelates
30 comprising these compositions preferentially accumulate in certain tissues, depending on the time after injection. In particular, 1,4,7,10 tetraazacyclododecane-1,4,7,10-tetra(methylenephosphonate monobutyl ester) has a high affinity for liver tissue and
35 the gastrointestinal tract (in that order). Chelates comprising this agent are thus suitable for liver imaging because of the lipophilic character imparted by the ester

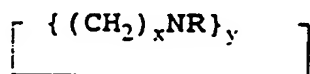
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functionality. Such agents are not metabolized, and eventually pass out of the body via the urine or feces.

While the monobutyl ester above appears well adapted for liver imaging, analogous alkyl esters have also been considered. Monopentyl esters are nearly as good for liver imaging, but monooctyl esters have the disadvantage of very low aqueous solubility. Monopropyl esters, on the other hand, may be used for liver imaging but are less efficient because a substantial portion of the agent is rapidly lost to the kidneys; monoisopropyl esters would behave similarly. Hence, the most preferred embodiment is that described above with monobutyl esters.

For use with NMR, compositions of the present invention must be chelated with a metallic element. While the element is preferably of the rare-earth series (preferably gadolinium), those skilled in the art will recognize that other metallic ions might also be useful for imaging. For example, other metal chelates (e.g., chelates of radionuclides or heavy metals) may be used for imaging by scintigraphy, x-radiation, and analogous imaging methods where changes in local tissue parameters can increase image contrast. Depending on the metal ion preferred for a particular contrast agent application, either triaza- or tetraaza- compounds of the present invention may be selected as chelators.

Chelators of the present invention have the formula



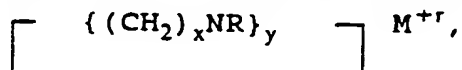
where

x is 2, 3 or a combination of p 2(s) and q 3(s) where $p + q = y$;
 y is 3 or 4;
 R is $(\text{CH}_2)_2\text{P}(=\text{O})\text{OR}^1$ or OR^2 ;
 R^1 is H or CH_3 ;

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R^2 is butyl, pentyl or hexyl; and
 z is 1 to 3.

In one important embodiment, this compound may be
 5 complexed with a metal to be a polyazamacrocyclic
 compound-metal complex having the formula



10 where

x is 2, 3 or a combination of p 2(s) and q 3(s)
 where $p + q = y$;

y is 3 or 4;

R is $(CH_2)_2 P(=O)OR^1 OR^2$;

15 R^1 is H or CH_3 ;

R^2 is butyl, pentyl or hexyl;

z is 1 to 3;

r is 2 or 3; and

M is a metal ion.

20

The y designation characterizes the compound as
 triazamacrocyclic or tetraazamacrocyclic. The x is
 preferably 2, although 3 is feasible under many
 circumstances. Combinations of p 2(s) and q 3(s) for x
 25 are of course readily produced but the total of $p + q$
 must be y for the number of units in the polyaza
 macrocycle. H or CH_3 for R^1 are believed equivalent in
 use.

30 In a preferred embodiment of either the compound or
 its metal complex y is 3, p is 1 and q is 2 or p is 2 and
 q is 1.

In another preferred embodiment of the compound or
 35 its metal complex, y is 4, p is 1 and q is 3, p is 2 and
 q is 2 or p is 3 and q is 1 and z is most preferably 1.
 n is preferably 2.

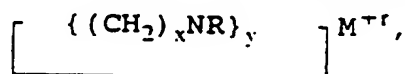
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In a more preferred embodiment x is 2, y is 4, z is 1, R¹ is H and R² is butyl.

In another preferred embodiment x is 2, y is 3, z is 1, R¹ is H and R² is butyl.

The M^{tr} is preferably a paramagnetic lanthanide, although other divalent or trivalent metal ions, including radionuclides and heavy metals, may also be so complexed.

In one important application, the present invention involves a method for enhancing a magnetic resonance image of a subject. This method comprises administering to the subject a polyazamacrocyclic compound-metal complex having the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)
where p + q = y;

y is 3 or 4;

R is (CH₂)₂P(=O)OR¹OR²;

R¹ is H or CH₃;

R² is butyl, pentyl or hexyl;

z is 1 to 3;

r is 3; and

M is gadolinium.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically illustrates the structure of NOTPME (where R is CH₂CH₃ and R¹ is H).

Figure 2 schematically illustrates the structure of DOTEPE.

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Figure 3 schematically illustrates the structure of DOTPMB.

5

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

EXAMPLE 1

Triazamacrocyclic Compounds

NOTPME Synthesis

10

Materials

1,4,7-triazacyclononane, paraformaldehyde, diethylphosphite, and activated carbon Darco G-60 were purchased from Aldrich Chemical Company. MgSO_4 was from Mallickrodt, sodium hydroxide, and benzene from J. T. Baker, and diethylether from Fisher Scientific. All chemicals were of highest purity and were used without further purification. Solutions of ZnCl_2 , GdCl_3 , MgCl_2 and CaCl_2 were standardized complexometrically.

20

Synthesis of NOTPME

1,4,7-Triazacyclononane (1.91 g, 14.71 mmol) and diethylphosphite (7.018 g, 16.94 mmol, 15% excess) were dissolved in 125 ml of benzene and heated to reflux. Anhydrous paraformaldehyde (1.727 g, 30% excess) was added in small portions to the above refluxing mixture while the benzene-water azeotropic mixture was removed by distillation. After the addition of paraformaldehyde was complete, the entire solution was boiled for 30 minutes and then evaporated to obtain a yellow viscous oil. The oil was dissolved in 150 ml anhydrous diethylether and dried with anhydrous MgSO_4 overnight. MgSO_4 , along with a white precipitate which formed, were filtered off and discarded. The filtrate was decolorized with activated carbon and filtered. The filtrate was evaporated in vacuum to obtain a viscous oil of 1,4,7-triazacyclononane-N,N',N''-tris(methylenephosphonate

35

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diethylester) (NOTPDE). Pure NOTPDE was obtained in 96% yield (9.21 g, 14.17 mmol) and was used for the synthesis of NOTPME (structure shown in Figure 1) without further purification. ¹H NMR data of NOTPDE in CDCl₃ (TMS at zero) are as follows: δ (ppm) : 1.33 (t, 18H, -CH₃), 2.97 (s, 12H, N-CH₂), 3.00 (d, 6H, P-CH₂), 4.13 (p, 12H, O-CH₂).

9.20 g of NOTPDE (14.15 mmol) was mixed with 2.50 g of NaOH in 9 ml H₂O and after 2 hours the entire reaction mixture was boiled until a clear solution was obtained (approximately 5 minutes). The solution was cooled to room temperature and was allowed to stand overnight. The crystals formed were filtered off from the viscous mother liquor using a pressure filter funnel with a coarse porosity grade filter disc. The crystals were washed once with cold absolute ethanol, three times with absolute ethanol-diethylether (1:1) mixture and finally with diethyl ether. The crystals of Na₃NOTPME were dried in dry nitrogen stream at 25°C for 2 hours. Traces of H₂O and ethanol were removed upon vacuum drying (10 mm Hg) NOTPME for 5 hours at 50°C. Pure NOTPME thus obtained were white crystals, very hygroscopic, readily soluble in H₂O, and fairly soluble in chloroform. The yield of pure NOTPME was 40.8% (3.24 g, 5.77 mmol).

¹H NMR (D₂O, HDO peak set as reference at 4.90 ppm), δ (ppm): 1.23 (t, 9H, -CH₃), 2.54 (s, broad, 6H, P-CH₂), 2.79 (s, broad, 12 H, N-CH₂), 3.91 (p, 6H, O-CH₂).

EXAMPLE 2

Tetraazamacrocyclic compounds

DOTEP Synthesis

DOTEP, shown in Figure 2, was prepared as follows. 2 ml of dichloroethylphosphine was slowly mixed with ice to form the corresponding ethylphosphinic acid. After

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warming to room temperature, 390 mg of 1,4,7,10-tetraazacyclododecane tetrahydrochloride (cyclen.4HCl) (Parrish Chem. Co., Ogden, Utah) was added and the mixture heated to boiling under a nitrogen atmosphere. A solution containing 157 mg of paraformaldehyde dissolved in 10 ml of 6M HCl was added at a rate of 0.5 ml/hr, while the mixture continued to reflux. The final mixture was refluxed an additional 4 hours then cooled to room temperature. This solution was concentrated under vacuum to a viscous oil, redissolved into 6 ml of water and loaded onto a DowEX 50Wx4 (hydrogen form) cation exchange column (7.5 ml bed volume). The column was washed to neutrality with water and the product eluted with 60 ml of 0.66 M HCl. The fractions containing DOTEPE were combined, evaporated, redissolved in absolute ethanol and evaporated to a white solid. This solid was dispersed into anhydrous ether, filtered off, pre-dried under nitrogen and dried under vacuum at 60-70°C to yield a white, very hygroscopic solid (360 mg, 44% yield). This solid was stored in sealed ampoules. Elemental analysis and potentiometry shows the solid to be DOTEPE.2HCl

EXAMPLE 3

Tetraazamacrocyclic compounds

DOTP Dibutyl Ester Synthesis

Tetraaza-12-crown-4.4HCl (1 g, 3.14×10^{-3} mol) was dissolved in water and the pH adjusted to 9.0 using 1M NaOH. The solvent was evaporated and the residue dried under vacuum for 1 hour. Formaldehyde (6.6 mL of 37% solution, 7.15 g, 0.24 mol) was added and the solution stirred for 30 minutes at room temperature. Dibutyl phosphite (5.10 mL of 96% purity, 0.025 mol) was then added and the reaction mixture stirred for 15 hours at room temperature (dipentyl and dihexyl phosphite are so used to produce dipentyl and dihexyl esters respectively). The resulting mixture consisted of two

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layers. The bottom layer was mostly excess formaldehyde, as indicated by ^{13}C NMR. The upper layer contained the product and excess phosphite. This layer was separated, concentrated and dried under vacuum for 1 hour. The resulting syrup was loaded onto a silica-gel column (2.5 x 11 cm). The excess phosphite was washed away with methylene chloride (250 mL). The product was eluted with 5% methanol in methylene chloride. 20 mL fractions were collected and monitored by TLC. The fractions containing the product were combined, concentrated, and dried under vacuum. A pale yellow oil was obtained in 75% yield (2.34 g). ^1H NMR (CDCl_3): 0.87 (t, $J=7.3$, 6H), 1.33 (m, $J=7.3$, 4H), 1.60 (p, $J=7.3$, 4H), 3.18 (br s, 4H), 3.39 (d, $J=8.5$, 2H), 4.03 (m, $J=7.3$, 6.1, 4H). ^{13}C NMR (CDCl_3): 11.3 (s), 16.5 (s), 30.3 (d, $J=5.9$), 47.3 (d, $J=148$), 50.0 (br s), 63.8 (d, $J=7.3$).

EXAMPLE 4

Tetraazamacrocyclic compounds

DOTP Monobutyl Ester (DOTPMB) Synthesis

The dibutyl ester was suspended in 1M KOH (20 mL). The mixture was stirred at 85°C for 17 hours and then at 106°C for 9 hours. The solvent was evaporated and the sample dried under vacuum for 1 hour. Methylene chloride (40 mL) was then added and the remaining solid KOH crushed as much as possible. The solvent was again evaporated and this procedure repeated another two times. The solvent was evaporated and the residue dissolved in methanol (60 mL). The mixture was filtered and then concentrated to a syrup under vacuum. Methylene chloride (80 mL) was added and the mixture filtered. The solvent was evaporated and the residue dried under vacuum to yield a white solid in 71% yield. ^{13}C NMR (D_2O ; ref. dioxane at 67.0 ppm): 13.5, 18.9, 32.9 (d, $J=5.9$), 50.7 (d, $J=140.6$), 51.5 (br s), 64.6 (d, $J=5.9$).

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EXAMPLE 5Biodistribution of Gd-DOTPMBComplexation and Biodistribution

5 A complex of DOTPMB (Figure 3) and Gd complex
 (0.012M based on metal, 2:1 ligand/metal ratio) was
 prepared and spiked with tracer quantities of Gd-159.
 Complexation was determined to be greater than 99% by
 standard analytical methods described in earlier reports.
10 Two Sprague-Dawley rats were then injected with the
 complex at a 0.05 mmol/kg level. The animals were
 sacrificed after 30 minutes and dissected for
 biodistribution data (Tables I and II); actual counts
 obtained from various tissues are shown in Table II. At
15 the end of this time period, an average of 58% of the
 injected dose was found in small intestine (see entry for
 SM INTES in Table I). A similar experiment performed
 with a third rat yielded 52% in small intestine (Tables
 III and IV); actual counts obtained from various tissues
20 are shown in Table IV. The bulk of the remaining
 activity in each case was eliminated via the renal system
 (Tables II and IV).

 In order for localization to occur in the small
25 intestine, the complex must first pass through the liver.
 Thus, since liver activity at the 30 minute time point
 (1%) was minimal (see e.g., Table I), the peak of liver
 localization passed within the prior 30 minutes. This is
 evident in an example of biodistribution 15 minutes after
30 administration of chelated tracer, which is documented in
 Tables VIII and IX. Although by 15 minutes the peak of
 liver localization had passed for mouse 1, with 4% in the
 liver and 88% in the small intestine, mouse 2 still had a
 significant liver concentration (66%) at the 15 minute
35 point. These animal models suggest that imaging within
 15 minutes after administration of chelated tracer will
 be necessary for best definition of the liver. The

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following test description supports that conclusion. Higher doses would, of course, lengthen the time of liver localization at concentrations sufficient to substantially enhance liver imaging.

5

Gamma Imaging of DOTPMB

A Sm(153)-DOTPMB complex was prepared as described above for gamma imaging of a Sprague-Dawley rat. Images were acquired at one minute intervals over a 16-minute period. The image sequence revealed concentration of the chelate in liver within one minute following injection. The complex is then rapidly transported from the liver to the stomach and small intestine. Tables V, VI and VII contain data taken at 1 hour, 24 hours and 72 hours after injection, showing movement of the agent from stomach and intestine to feces.

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TABLE I

TIME: 30 Minute Biodistribution

DATE LIGAND METAL COMMENTS
 5 8/6/91 DOTPMB-K, Gd-159, 99% Complexation
 4:1, LIG:MET Molar Ratio, (Metal = $3 \times 10^{-4} \text{M}$)

%DOSE/GRAM				
	RAT 1	RAT 2	AVERAGE	+/-
10 WEIGHT	216.72	228.17	222.45	8.096
BONE	0.019	0.006	0.01	0.009
TAIL	0.077	0.068	0.07	0.007
LIVER	0.114	0.069	0.09	0.032
15 KIDNEY	0.169	0.126	0.15	0.031
SPLEEN	0.011	0.021	0.02	0.007
MUSCLE	0.007	0.005	0.01	0.001
BLOOD	0.021	0.017	0.02	0.003
HEART	0.014	0.000	0.01	0.010
20 LUNG	0.126	0.023	0.07	0.073
BRAIN	0.002	0.003	0.00	0.000
0.000 = NO ACTIVITY DETECTED				
DOSE				
25 BONE	0.281	0.106	0.193	0.124
TAIL	0.212	0.191	0.201	0.015
LIVER	1.234	0.755	0.994	0.339
KIDNEY	0.392	0.299	0.346	0.066
SPLEEN	0.009	0.011	0.010	0.002
MUSCLE	0.658	0.506	0.582	0.108
30 BLOOD	0.299	0.250	0.274	0.035
HEART	0.011	0.000	0.006	0.008
LUNG	0.158	0.029	0.094	0.091
BRAIN	0.004	0.004	0.004	0.001
STOMACH	0.574	0.530	0.552	0.031
35 SM INTES	80.304	36.405	58.354	31.041
LG INTES	0.591	0.433	0.512	0.112

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TABLE II (see legend for Table I)

DATA	COUNTS	ORGAN	N ENTER WT	BCKG COR	%DOSE/G	% DOSE
1		Std A	X Rat 1 Wt	0		
1		Std B	X 216.72	0		
2	414838	Std C	av 414404.5	414405		
3	480	Bone	0.60	47	1.87E-02	2.81E-01
4	1311	Tail	2.74	878	7.73E-02	2.12E-01
5	5546	Liver	10.86	5113	1.14E-01	1.23E+00
6	2058	Kidney	2.32	1625	1.69E-01	3.92E-01
7	469	Spleen	0.75	36	1.14E-02	8.57E-03
8	478	Muscle	1.52	45	7.06E-03	6.58E-01
9	576	Blood	1.62	143	2.12E-02	2.99E-01
10	481	Heart	0.82	48	1.40E-02	1.15E-02
11	1088	Lung	1.25	655	1.26E-01	1.58E-01
12	449	Brain	1.62	16	2.31E-03	3.74E-03
13	2811	Stomach		2378		5.74E-01
14	333215	Sm Intes		332782		8.03E+01
15	2883	Lg Intes		2450		5.91E-01
16	150310	Urine		149877		3.62E+01
17	400	Urine		0		0.00E+00
18	428	Urine		0		0.00E+00
19	443	BKG	228.17	WT Rat 2		
20	451	Bone	0.67	18	6.30E-03	1.06E-01
21	1225	Tail	2.82	792	6.77E-02	1.91E-01

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DATA	COUNTS	ORGAN	N ENTER WT	BCKG COR	%DOSE/G	% DOSE
22	3561	Liver	10.96	3128	6.89E-02	7.55E-01
23	1674	Kidney	2.38	1241	1.26E-01	2.99E-01
24	480	Spleen	0.53	47	2.12E0-02	1.12E-02
25	469	Muscle	1.66	36	5.16E-03	5.06E-01
26	559	Blood	1.80	126	1.68E-02	2.50E-01
27	430	Heart	0.85	0	0.00E+00	0.00E+00
28	554	Lung	1.28	121	2.27E-02	2.91E-02
29	452	Brain	1.55	19	2.88E-03	4.46E-03
30	2629	Stomach		2196		5.30E-01
	151297	Sm Intes		150864		3.64E+01
	2229	Lg Intes		1796		4.33E-01
	116731	Urine		116298		2.81E+01
	901	Urine		468		1.13E-01
	423	Urine		0		0.00E+00
	424	BKG		0		

BKG AVG = 434

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TABLE III

TIME: 30 Minute Biodistribution

DATE LIGAND METAL COMMENTS
 5 8/6/91 DOTPMB-K, Gd-159, 99% Complexation
 4:1, LIG:MET Molar Ratio, (Metal = $3 \times 10^{-4}M$)

		%DOSE/GRAM RAT 3
	WEIGHT: 235.14	
10	Bone	0.006
	Tail	0.013
	Liver	0.016
	Kidney	0.121
	Spleen	0.000
15	Muscle	0.000
	Blood	0.000
	Heart	0.000
	Lung	0.009
	Brain	0.004
20	0.000 = No Activity Detected	
	Bone	0.109
	Tail	0.038
25	Liver	0.196
	Kidney	0.303
	Spleen	0.000
	Muscle	0.026
	Blood	0.000
30	Heart	0.000
	Lung	0.012
	Brain	0.005
	Stomach	0.012
	Sm Intes	51.549
35	Lg Intes	2.232

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TABLE IV (see legend for Table III)

	DATA	COUNTS	ORGAN	N ENTER WT	BCKG COR	%DOSE/G	% DOSE
5	1		Std A	X Rat 1 Wt	0		
	1		Std B	X 235.14	0		
10	2	414367	Std C	av 413946	413946		
	3	439	Bone	0.69	18	6.30E-03	1.09E-01
	4	578	Tail	2.95	157	1.29E-02	3.79E-02
15	5	1232	Liver	11.96	811	1.64E-02	1.96E-01
	6	1675	Kidney	2.51	1254	1.21E-01	3.03E-01
20	7	418	Spleen	0.66	0	0.00E+00	0.00E+00
	8	423	Muscle	1.91	2	2.53E-04	2.56E-02
	9	412	Blood	1.84	0	0.00E+00	0.00E+00
25	10	417	Heart	0.85	0	0.00E+00	0.00E+00
	11	470	Lung	1.25	49	9.47E-03	1.18E-02
30	12	442	Brain	1.28	21	3.96E-03	5.07E-03
	13	470	Stomac h		49		1.18E-02
	14	213806	Sm Intes		213385		5.15E+01
35	15	9661	Lg Intes		9240		2.23E+00
	16	102818	Urine		102397		2.47E+01
40	17	12520	Urine		12099		2.92E+00
	18	447	Urine		26		6.28E-03
45	19	421	BKG				

BKG AVG = 421

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TABLE V

One Hour Biodistribution, Imaged Rat

TIME: One Hour biodistribution

DATE LIGAND METAL COMMENTS
 8/8/91 DOTPMB-K, D. Sm=153 99% Complex
 4:1, LIG:MET Molar Ratio (Metal = $3 \times 10^{-4} \text{M}$)

	% DOSE/GRAM	% DOSE
WEIGHT: 263.82		
Bone	0.004	0.067
Tail	0.038	0.093
Liver	0.014	0.144
Kidney	0.084	0.245
Spleen	0.016	0.011
Muscle	0.001	0.078
Blood	0.001	0.019
Stomach		45.144
Small Int		31.408
Lrg Int		0.003

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TABLE VI

24 Hour Biodistribution, Imaged Rat

TIME: 24 Hour biodistribution

5 DATE LIGAND METAL COMMENTS
 8/8/91 DOTPMB-K Sm=153 99% Complex
 4:1, LIG:MET Molar Ratio (Metal = $3 \times 10^{-4} \text{M}$)

		% DOSE/GRAM	% DOSE
10	WEIGHT: 263.82		
	Bone	0.008	0.146
	Tail	0.015	0.036
	Liver	0.010	0.106
	Kidney	0.154	0.451
15	Spleen	0.029	0.020
	Muscle	0.017	1.883
	Blood	0.000	0.004
	Stomach		0.053
	Sml Int		2.109
20	Sml Int		19.611
	Lrg Int		8.351
	Feces		35.981
	Urine		2.799
25	Paper		0.126

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TABLE VII

Rat Injected: 8/8/91

Time: 72 hour biodistribution

5 Date: Ligand Metal Comments
 8/12/91 DOTPMB-K Sm-153 99% Complex
 4:1, LIG:MET MOLAR RATIO (Metal = $3 \times 10^{-4} \text{M}$)

		%DOSE/GRAM	% DOSE
10	WEIGHT: 269.58		
	Bone	0.014	0.219
	Tail	0.002	0.005
	Liver	0.002	0.030
	Kidney	0.019	0.054
15	Spleen	0.000	0.000
	Muscle	0.000	0.000
	Blood	0.000	0.000
	Feces		5.832
	Feces		5.467
20	Feces		2.464
	Urine		0.077
	Bladder		0.242

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TABLE VIII

File=BTY15STD Summary Standardized Data Mouse #2
 (15 minute biodistribution), 99% complex, 25.0 UL dose
 Ca added to complex at 1;1 molar, lig: Ca

DATE LIGAND METAL COMMENTS
 10/30/91 DOTPME-K Sm-15 #08-07-91

2:1, Lig:Met Molar Ratio, (Metal=3x10⁻⁴M), pH 7-8

%DOSE/GRAM			
	MOUSE 1	MOUSE 2	AVERAGE
WEIGHT	15.854	10.702	13.278
BONE	0.171	3.169	1.670
TAIL	1.374	83.296	42.335
LIVER	3.979	66.441	35.210
KIDNEY	0.889	14.568	7.728
SPLEEN	1.260	0.831	1.046
MUSCLE	0.173	1.437	0.805
BLOOD	43.105	3.743	23.424
HEART	0.472	2.418	1.445
LUNG	0.697	2.735	1.716
BRAIN	0.023	0.139	0.081
TUMOR	0.648	4.844	2.746
STOMACH	14.272	4.343	9.308
SMALL INT.	88.504	40.343	64.423
LARGE INT.	3.320	2.298	2.809
URINE	0.000	0.000	0.000
BODY 1	0.408	1.637	1.022
BODY 2	0.148	5.594	2.871
*MOUSE 2 DID NOT BECOME ACTIVE AFTER ANESTHESIA, HOWEVER ALIVE***			

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TABLE IX

%DOSE			
	MOUSE 1	MOUSE 2	
5	BONE	0.149	2.100
	LIVER	3.379	37.732
	KIDNEY	0.199	3.610
	SPLEEN	0.095	0.039
	MUSCLE	1.179	6.615
10	BLOOD	44.420	2.604
	HEART	0.033	0.157
	LUNG	0.090	0.210
	BRAIN	0.010	0.054
	TUMOR	0.062	0.237
15	STOMACH	3.565	0.586
	SMALL INT.	86.734	27.837
	LARGE INT.	2.994	1.145
	URINE	12.857	0.007
	BODY 1	2.294	6.890
20	BODY 2	0.585	17.147

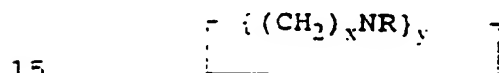
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Changes may be made in the construction, operation and arrangement of the various parts, elements, steps and procedures described herein without departing from the concept and scope of the invention as defined in the following claims.

CLAIMS

1. A polyazamacrocyclic compound or a salt thereof, the compound having the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)
where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z\text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

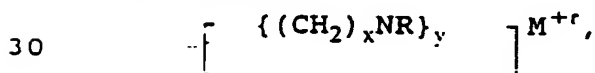
R^1 is H or CH_3 ;

R^2 is $\text{C}_n\text{H}_{1+2n}$;

z is 1 to 3; and

n is 4 to 6.

2. A polyazamacrocyclic compound-metal complex having the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)

where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z\text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

R^1 is H or CH_3 ;

R^2 is $\text{C}_n\text{H}_{1+2n}$;

z is 1 to 3;

-24-

r is 2 or 3;
M is a metal ion; and
n is 4 to 6.

5

3. The compound of claim 1 where y is 3.

10

4. The compound of claim 1 where y is 4.

5. The compound of claim 1 where y is 3 and x is 2.

15

6. The compound of claim 1 where y is 4 and x is 2.

7. The complex of claim 2 where y is 3.

20

8. The complex of claim 2 where y is 4.

9. The complex of claim 2 where y is 3 and x is 2.

25

10. The complex of claim 2 where y is 4 and x is 2.

30

11. The compound of claim 1 where y is 3, p is 1 and q is 2 or p is 2 and q is 1.

35

12. The complex of claim 2 where y is 3, p is 1 and q is 2 or p is 2 and q is 1.

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13. The compound of claim 1 where y is 4, p is 1 and q is 3, p is 2 and q is 2 or p is 3 and q is 1.

5 14. The complex of claim 2 where y is 4, p is 1 and q is 3, p is 2 and q is 2 or p is 3 and q is 1.

10 15. The compound of claim 1 where z is 1.

16. The complex of claim 2 where z is 1.

15 17. The compound of claim 1 where R^2 is C_4H_9 .

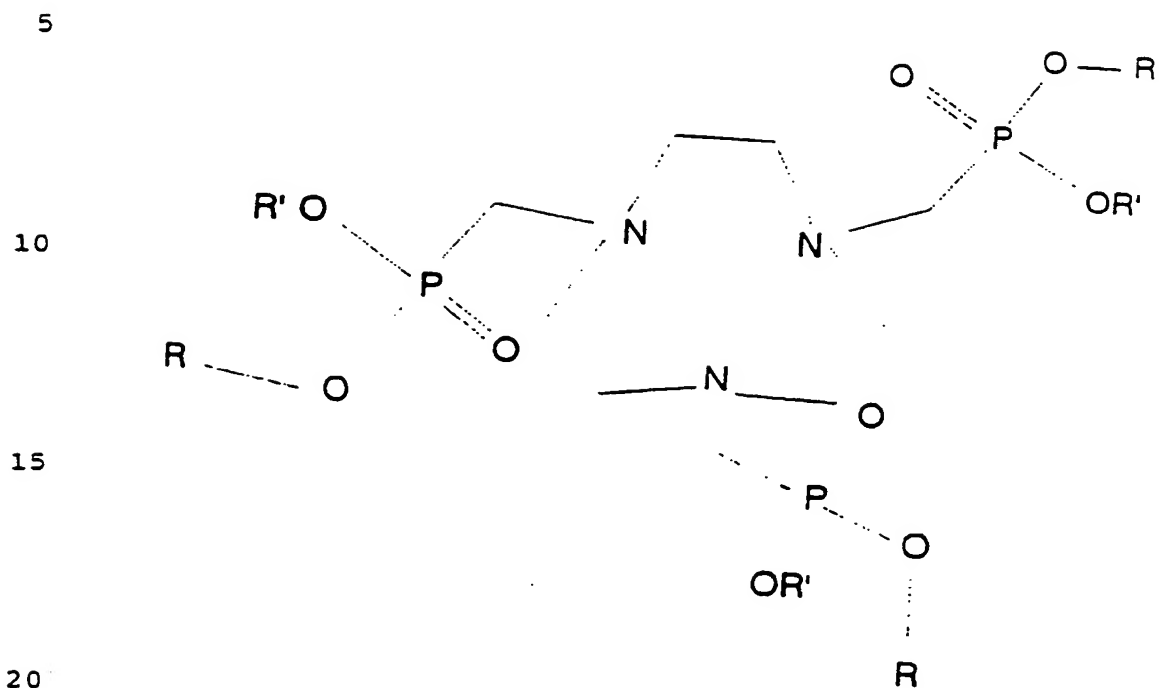
18. The complex of claim 2 where R^2 is C_4H_9 .

20 19. The complex of claim 2 where M is a lanthanide element.

25 20. The complex of claim 2 where M^{+r} is Gd^{+3} .

-26-

21. A compound or salt thereof, the compound having the formula:



where R is C_nH_{1+2n} ;

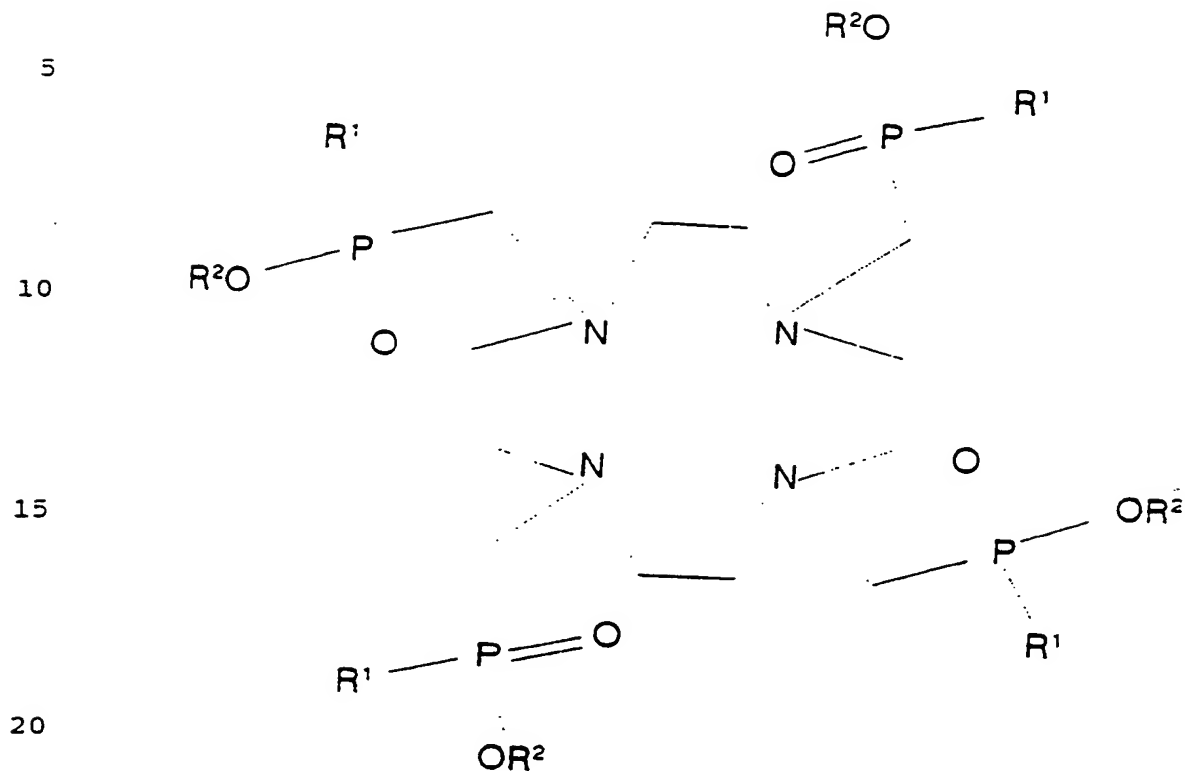
n is 4 to 6; and

R' is H.

25

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22. A compound or salt thereof, the compound having the formula:



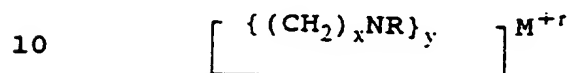
25 where R^1 is OC_nH_{1+2n} ;
 n is 4 to 6; and
 R^2 is H.

30 23. The compound of claim 21 or 22 where n is 4.

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24. A method for increasing contrast of nuclear magnetic resonance images of a patient, the method comprising

administering to a patient needing nuclear magnetic resonance imaging a polyazamacrocyclic compound or a salt thereof, the compound having the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)

where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

R^1 is H or CH_3 ;

R^2 is $\text{C}_n\text{H}_{1+2n}$;

n is 4 to 6;

z is 1 to 3;

r is 2 or 3; and

M is a metal ion; and

obtaining a magnetic resonance image of the patient.

25. The method of claim 24 wherein x is 2, y is 3, z is 1, and n is 4.

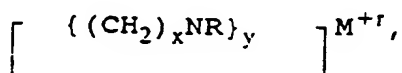
26. The method of claim 24 wherein x is 2, y is 4, z is 1, and n is 4.

27. The method of claim 24 wherein M is a lanthanide element.

28. The method of claim 24 wherein M is Gd^{+3} .

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29. A method for enhancing an x-ray image of a subject, the method comprising administering to the subject a polyazamacrocyclic compound-metal complex having the formula



10 where

x is 2, 3 or a combination of p 2(s) and q 3(s)
where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

15 R^1 is H or CH_3 ;

R^2 is $\text{C}_n\text{H}_{1+2n}$;

n is 4 to 6;

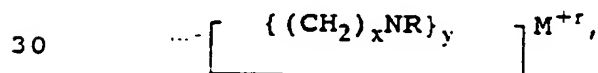
z is 1 to 3;

M is a heavy metal; and

20 r is 2 or 3; and

obtaining a magnetic resonance image of said subject.

30. A method for enhancing a scintigraphic image of a
25 subject, the method comprising administering to the
subject a polyazamacrocyclic compound-metal complex
having the formula



30 where

x is 2, 3 or a combination of p 2(s) and q 3(s)
35 where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

R^1 is H or CH_3 ;

R^2 is $\text{C}_n\text{H}_{1+2n}$;

40 z is 1 to 3;

-30-

M is a radionuclide metal; and
 r is 2 or 3; and
 obtaining a scintigraphic image of said subject.

5

31. The method of claim 30 wherein M is samarium 153.

10

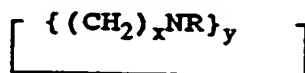
32. The method of claim 29 or 30 wherein x is 2, y is 3,
 z is 1, and n is 4.

15

33. The method of claim 29 or 30 wherein x is 2, y is 4,
 z is 1, and n is 4.

20

34. A polyazamacrocyclic compound or a salt thereof, the
 compound having the formula



where

25

x is 2, 3 or a combination of p 2(s) and q 3(s)
 where $p + q = y$;

y is 3 or 4

R is $(\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

R^1 is H;

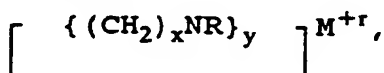
R^2 is C_4H_9 ; and

30

z is 1 to 3.

35. A polyazamacrocyclic compound-metal complex having
 the formula

35



where

-31-

x is 2, 3 or a combination of p 2(s) and q 3(s)
where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z\text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

5 R^1 is H;

R^2 is C_4H_9 ;

z is 1 to 3;

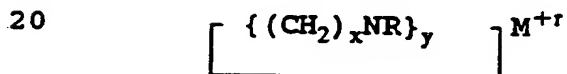
r is 2 or 3; and

M is a metal ion.

10

36. A method for increasing contrast of nuclear magnetic resonance images of a patient, the method comprising

15 administering to a patient needing nuclear magnetic resonance imaging a polyazamacrocyclic compound or a salt thereof, the compound having the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)
25 where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z\text{P}(=\text{O})\text{OR}^1\text{OR}^2$;

R^1 is H;

R^2 is C_4H_9 ;

30 z is 1 to 3;

r is 2 or 3; and

M is a metal ion; and

obtaining a magnetic resonance image of the patient.

35

37. The complex of claim 35 where M^{+r} is Gd^{+3} .

38. The method of claim 36 where M^{+r} is Gd^{+3} .

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39. A compound or salt thereof, the compound having the formula:

5

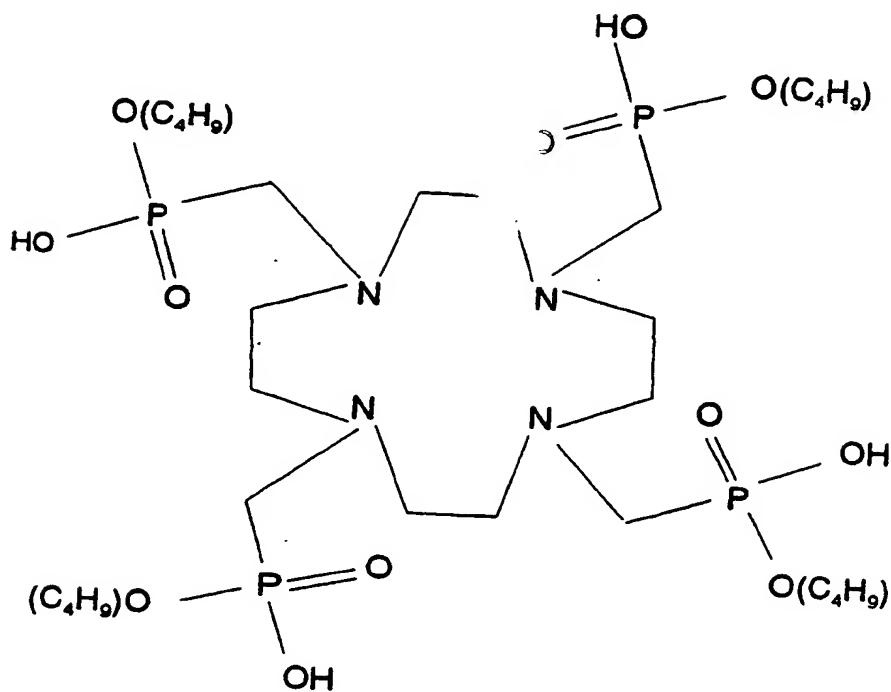
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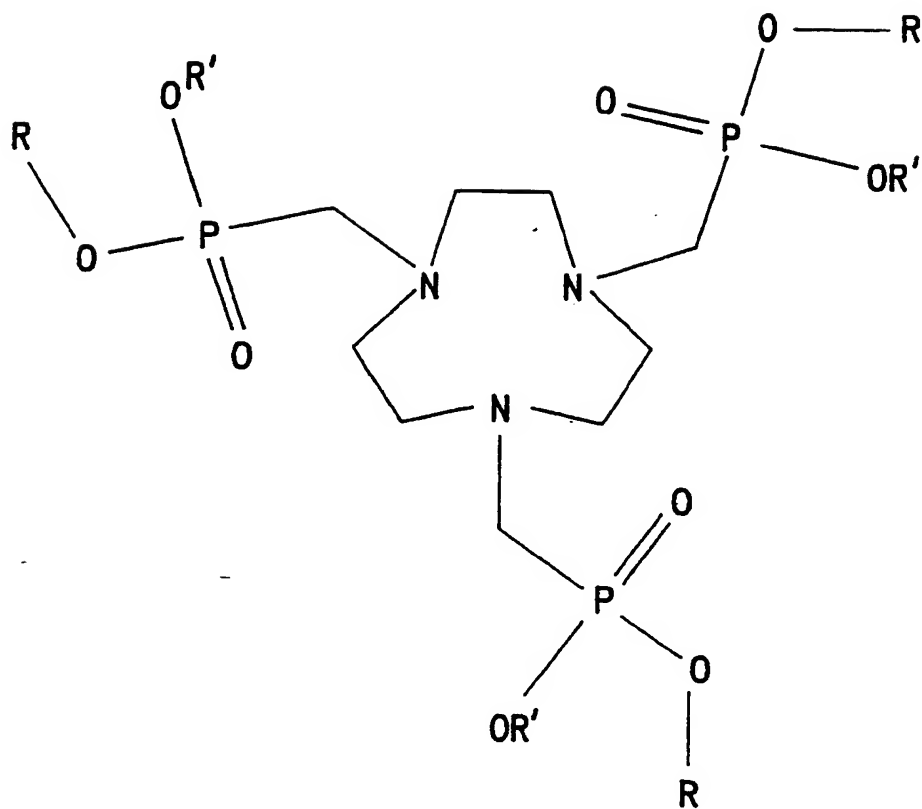


FIG.1

2/3

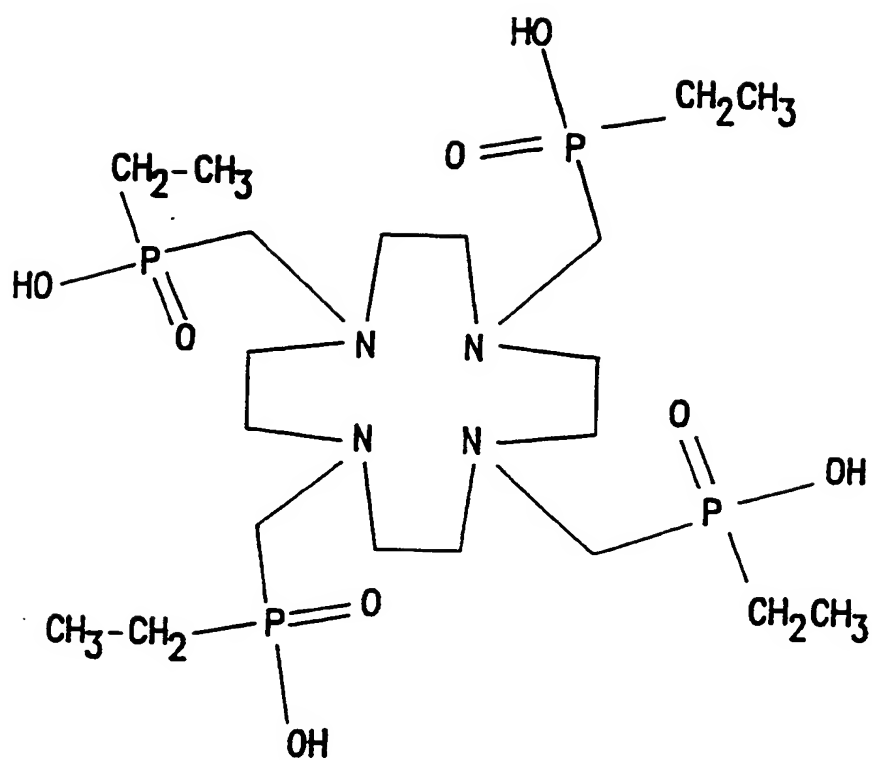


FIG.2

3/3

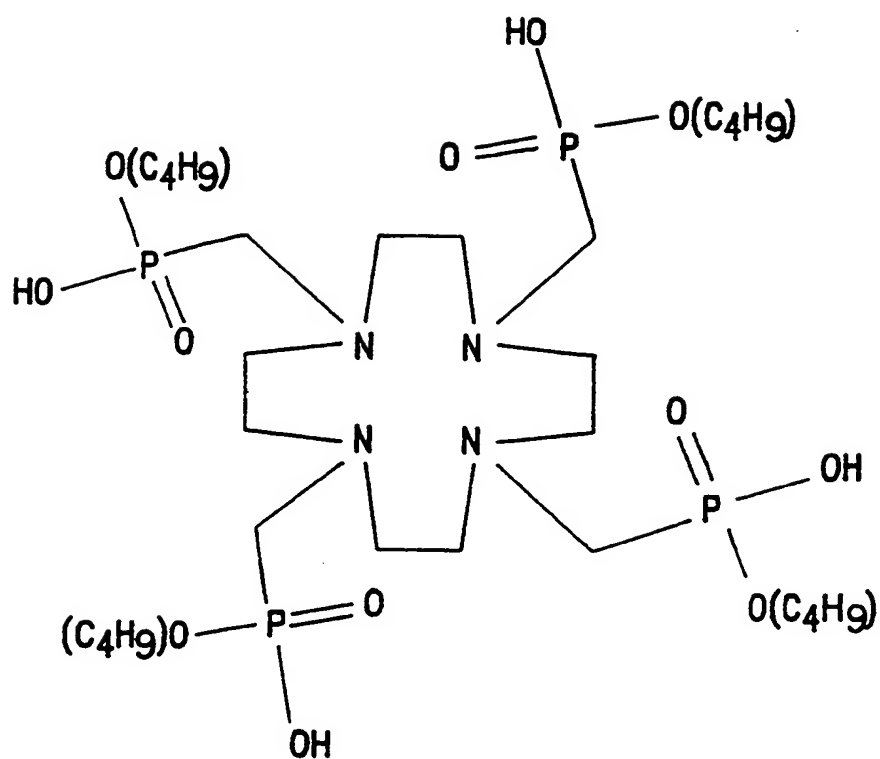


FIG.3

INTERNATIONAL SEARCH REPORT

International Application

PCT/US 93/06158

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)*

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07F9/6515; A61K49/00; C07F9/6524

II. FIELDS SEARCHED

Minimum Documentation Searched?

Classification System

Classification Symbols

Int.Cl. 5 C07F ; A61K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched*

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
------------	--	-------------------------------------

X	WO, A, 9 208 725 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 29 May 1992 see page 5, line 1 - line 27; claims 17, 18	1-39
---	--	------

A	JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS 1991, LETCHWORTH GB pages 1252 - 1253 ISTVAN LAZAR 'N, N', N'-Tris(methoxymethyl) -1,4,7-triazacyclononane: a New Synthetic Tool for the Synthesis of Tris-N-substituted 1,4,7-Triazacyclononane Derivatives ¹	
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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

22 SEPTEMBER 1993

Date of Mailing of this International Search Report

01. 10. 93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

BESLIER L.M.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9306158
SA 76662

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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22/09/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9208725	29-05-92	AU-A- 9077091	11-06-92
		CN-A- 1066073	11-11-92
		EP-A- 0558661	08-09-93
		US-A- 5188816	23-02-93

EP FORM P0019

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

